Ultrastructural studies on the spermatogenesis of the African mussel *Perna perna* (Bivalvia: Mytilidae)

Estudio ultraestructural de la espermatogénesis del mejillón africano *Perna perna* (Bivalvia: Mytilidae)

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ABSTRACT

Transmission electron microscopy of spermiogenesis in gonads of *Perna perna* (Mytilidae) was carried out to study the different developmental stages and the structure of mature sperm. Samples of mussels originated from a population living at Cap Ghir (southwestern Morocco) and were collected from December 1999 to June 2000 at several periods of their sexual cycle. Numerous acini, surrounded by a connective tissue containing vesicular cells, constituted the male gonad of *P. perna*. Groups of spermatogonia, spermatocytes, and spermatids, interconnected by intercellular bridges, were found in these acini. Proacrosomal vesicles and a short flagellum developed early in spermatogonia. Numerous Sertoli-like cells were distributed among spermatogonia, nearest the inner wall of each acinus. The reduced cytoplasm of primary and secondary spermatocytes mainly contained mitochondria and proacrosomal vesicles. Spermatogenesis consisted of a progressive condensation of the chromatin and a reduction in size of the nucleus, the migration of the proacrosomal granule from the base of the nucleus to its apex to become the acrosome, and the elaboration of the flagellum by the distal centriole. The nucleus of the mature spermatozoon was barrel shaped and contained a completely condensed chromatin. The funnel shaped acrosome showed two types of electron-dense materials in the basal and apical parts, and a more lucent zone in the subacrosomal invagination and the center of the premembranoid sleeve. An axial rod could be observed from the apex of the acrosome up to the base of the nucleus. The length of the nucleus in the spermatozoon, the premembranoid sleeve, and the presence of three types of materials in the acrosome might be additional criteria to separate the Moroccan populations of *P. perna* from other Mytilidae and, in particular, from those of *Mytilus galloprovincialis* which also lives along Moroccan coasts.

RESUMEN

Las gónadas de *Perna perna* (Mytilidae) se examinaron en microscopía electrónica de transmisión (TEM) con el fin de estudiar los estadios de desarrollo y la estructura del esperma maduro. Las muestras de mejillones se originaron de una población asentada en Cap Ghir (suroeste de Marruecos) y fueron colectadas entre diciembre de 1999 y junio de 2000 en varios momentos de su ciclo sexual. La gónada macho de *P. perna* se compone de numerosos ácinos, rodeados por un tejido conectivo que contiene células vesicu-

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losas. Grupos de espermatogonias, espermatocitos y espermatidias, vinculados por puentes intercelulares, fueron encontrados en estos ácinos. Vesículas proacrosomales y un flagelo corto se desarrollaron temprano en las espermatogonias. Numerosas células tipo Sertoli estaban distribuidas en las espermatogonias, junto a la pared interna de cada ácino. El citoplasma reducido de espermatocitos primarios y secundarios contenía principalmente mitocondrias y vesículas proacrosómicas. La espermiogénesis consistió en una progresiva condensación de la cromatina y reducción del tamaño del núcleo, en la migración del gránulo proacrosomal desde la base del núcleo hasta su ápice en donde forma el acrosoma y en la elaboración del flagelo por el centriolo distal. El núcleo del espermatozoo maduro tiene forma de barril y contiene una cromatina completamente condensada. El acrosoma, en forma de embudo, presenta dos tipos de materiales densos frente a los electrones de las partes basal y apical y una zona más clara en la invaginación subacrosomal y en el centro de la manga premembranóide. Una varilla axial se puede observar entre el ápice del acrosoma y la base del núcleo. La longitud del núcleo del espermatozoo, la manga premembraníode y la presencia de tres tipos de material en el acrosoma pueden resultar criterios adicionales para diferenciar las poblaciones marroquíes de *P. perna* de otros Mytilidae y particularmente de aquellas de *Mytilus galloprovincialis*, que también vive en la costa marroquí.

KEY WORDS: *Perna perna*, spermatogenesis, *Mytilidae*, ultrastructure.
PALABRAS CLAVE: *Perna perna*, espermatogénesis, *Mytilidae*, ultraestructura.

INTRODUCTION

In Bivalvia, the morphology of the spermatozoon gives information on the mode of fertilization (in water, or in the mollusc) (Franzen, 1955) and on the systematic position of taxa (Healy, Keys and Daddow, 2000). As the morphology of this gamete is well conserved in several taxa, it is considered to be a good indicator of phylogenetic relationships between species, in particular when these come close to each other (Popham, 1979; Healy *et al.*, 2000). A good example is the family Mytilidae, as the characteristics of the spermatozoon vary remarkably between genera (Kafanov and Drozdov, 1998).

Contrary to studies which have been carried out on the spermatogenesis of numerous mytilids, little information on the developmental cell stages of the male line in *Perna perna* is available in literature. The fine structure of mature spermatozoa of this mussel was already studied by Bourcart, Lavallard and Lubet (1965), or by Bernard and Hodgson (1985), while aspects of spermiogenesis in *P. perna* were first described by Bernard and Hodgson (1985). By contrast, to our knowledge, there are no published reports on the first cell stages of spermatogenesis. Therefore, the aim of this work was to describe the fine structure of the developmental cell stages which succeeded in the spermatogenesis of this mussel and, in particular, during the formation of the acrosome, as this organelle has a direct implication on the fertilization of the oocyte.

As other populations of *P. perna* live worldwide under tropical conditions (see the review by Hicks, McMahon and Ingrao, 2001), it is interesting to detect the variations which may exist in the morphology of spermatozoa when they originate from different communities of mussels, as the fine structure of these male cells has already been studied in Brazilian (Bourcart *et al.*, 1965) as well as in South African (Bernard and Hodgson, 1985) populations of *P. perna*. Consequently, the second aim of this work was to compare spermatozoon morphologies between

the abovementioned populations of *P. perna* and the Moroccan mussels, as *P. perna* is present here at the northern limit of its distribution along the Atlantic coasts of Africa.

Finally, as the Moroccan mussels are abundantly consumed by local people, a project to develop the breeding of this mollusc for commercial purposes has been set up in Morocco (BERRAHO, 1998). However, to realize this project, more detailed information on the local reproduction of *P. perna* was necessary. Several studies of our team were already carried out on the growth, ecology, reproduction, and reserve strategy of this species (ID HALLA, BOUHAIMI, ZEKHNINI, NARBONNE, MATHIEU AND MOUKRIM, 1997; NAJIMI, BOUHAIMI, DAUBEZE, ZEKHNINI, PELLERIN, NARBONNE AND MOUKRIM, 1997; KAAYA, NAJIMI, RIBERA, NARBONNE AND MOUKRIM, 1999; MOUKRIM, KAAYA, NAJIMI, ROMÉO AND GNASSIA-BARELLI, 2000). The work reported in the present study completes these first papers and gives information on the development of the male line in this mussel.

MATERIALS AND METHODS

Samples of five male mussels each (3-4 cm long, 8-9 g in weight) were collected in December 1999, January, March, June, and July 2000 from the mid-tide level at Cap Ghir (50 km north from Agadir town). The choice of these months for mussel sampling was based on the different sexual stages observed by ID HALLA *ET AL.* (1997) in the same population of *P. perna*: December (II stage), January (IIIA stage), March (IIIB and IIIC stages), June (IIIC and IIID stages), and July (IIIC and IIID stages). Small portions of male gonad (1-3 mm^3 each) were fixed for 60 min in 2% glutaraldehyde (in 0.4 M sodium cacodylate buffer, pH 7.2) at 4°C. The tissue was then washed in 0.4 M cacodylate buffer (3 x 10 min) and postfixed for 90 min in 1% tetroxide osmium (in 0.4 M cacodylate buffer) at 4°C. After dehydration through a gradual ethanol series, the tissues were directly embedded in Epon resin at 37°C for 60 min and were subsequently placed at room temperature during 12 hours for polymerization. Semi-thin sections of each gonad portion were stained at room temperature with 0.5% toluidine blue in 2.5% Na_2CO_3. Ultrathin sections were collected on copper grids and were stained for 20 min with uranyl acetate, followed by lead citrate for 5 min. Sections were examined using a Siemens 102 electron microscope.

The length and the width of each cell stage were also measured (10 cells at least per stage). Individual values recorded for each measurement and each cell stage were averaged.

RESULTS

Each gonad was constituted by many acini, surrounded by connective tissue and an external ciliated epithelium (Fig. 1A). From the internal wall of each acinus to the lumen, all developmental stages were present (Fig. 1B). Groups of spermatogonia are positioned nearest the wall of each acinus, groups of spermatocytes and spermatids were located closer to the acinus lumen, whereas the flagella of mature sperm were confined to the central lumen (Fig. 1B). All acini emptyed into spermatic ducts, each being lined by an internal ciliated, columnar epithelium. Vesicular cells, containing two types of granules, were distributed within the interacinar connective tissue (Fig. 1A).

Spermatogonia: Two types of spermatogonia (Fig. 2 A,B) were observed in the gonad of *P. perna*. The A spermatogonia (primordial cells) were large cells (16.6 x 5.9 μm in size). Their nucleus was often ovoid (6.3 x 4.0 μm), each with a single 1.6-μm nucleolus, and their translucent cytoplasm contained an extensive rough endoplasmic reticulum (RER), numerous mitochondria, and a Golgi apparatus. B spermatogonia are smaller cells (7.2 x 3.0 μm) and their nuclei (4.2 x 2.3 μm) had a well-developed euchromatin and sometimes a

visible nucleolus. Their dark cytoplasm contained numerous mitochondria, RER cisternae, and proacrosomal vesicles derived from the Golgi complex. These B spermatogonia had an irregularly-shaped membrane and intercellular bridges were frequently observed. In some B cells, a short flagellum elaborated by the distal centriole, at the base of the nucleus, could be seen. When the nuclei of B cells underwent metaphase (Fig. 3A), these spermatogonia became smaller (4.6 x 4.1 μm). Numerous Sertoli-like cells were distributed among spermatogonia, nearest the wall of each acinus. Each of these elongated (10.5 x 3.0 μm) and regularly-shaped cells contained an ovoid nucleus with chromatin condensed along the nuclear envelope. Numerous cytoplasmic processes in contact with close spermatogonia could be observed. The cytoplasm of these Sertoli-like cells contained numerous electron-dense inclusions, mitochondria, several lipid inclusions, and myelin-like figures (Fig. 2 A,B, and Fig. 3A).

Spermatocytes: The primary spermatocytes (Fig. 3B, C) were arranged in groups of 2-4 cells each, with intercellular bridges. When these cells were in prophase (pachytene stage), their nuclei were similar in shape to those of B spermatogonia. However, their nuclear envelope was thicker, with a clearly wider perinuclear cisterna. The small size (6.7 x 3.8 μm) of their cytoplasm increased the nucleo-cytoplasmic ratio. These cells contained the same organelles as described for B spermatogonia. A short flagellum was often observed (Fig. 3B, C).

The secondary spermatocytes were scarcely visible, due to the rapidity of the second meiotic division. These cells were irregular in shape (5.7 x 4.3 μm) and their rounded nuclei (a mean of 3.3-3.4 μm in diameter) contained chromatin forming a dense network at metaphase stage. Mitochondria and proacrosomal vesicles were mainly present in their reduced cytoplasm (Fig. 3D).

Spermatids: Their development within the gonad can be arbitrarily divided into four stages, from A the youngest, to D the oldest. A spermatids (Fig. 4A) were irregular in shape (7.4 x 6.6 μm) and their round nuclei (4 μm) contained scattered heterochromatin. Grouped mitochondria and proacrosomal vesicles close to the Golgi apparatus could be seen in their cytoplasm. B spermatids (Fig. 4B) were smaller (4.1 x 3.2 μm) than A cells and their nuclei were more reduced (2.5 x 1.9 μm), eccentrically positioned, and contained a more condensed heterochromatin. A proacrosomal granule, resulting from the fusion of the corresponding vesicles, was present in their cytoplasm. In C spermatids (Fig. 4C), the nuclei became rounded (2.2 x 1.9 μm) and the nuclear chromatin was continuously condensing, thus showing a patchwork pattern. The proacrosomal granule migrated from the basal part of the nucleus to the cell apex and progressively modified its shape. Its basal part, towards the nucleus, became flattened, whereas an invagination in the granule formed the subacrosomal zone, containing a material less electron-dense than that observed in the granule. Spherical mitochondria set up at the base of the nucleus and surrounded the centrioles. The nuclei of the oldest spermatids (Fig. 4D) became elongated, were completely condensed, and showed two invaginations, the first at the apex of the nucleus and the second at its base near the distal centriole. The proacrosomal granule progressively became funnel-like and turned into an acrosome containing a dense material. In several spermatids, a premembranoid sleeve was sometimes observed in the apical part of the acrosome. The flagellum showed gradual structural changes during the differentiation of spermatids. It is elaborated by the distal centriole and was surrounded by a ring of five mitochondria at the base of the nucleus. The distal and proximal centrioles were situated in perpendicular position (Fig. 4D).

Spermatozoa: In the 5.3 μm long head, the nucleus (1.35 μm in size) was barrel-like and was completely condensed. The very long acrosome was funnel-like

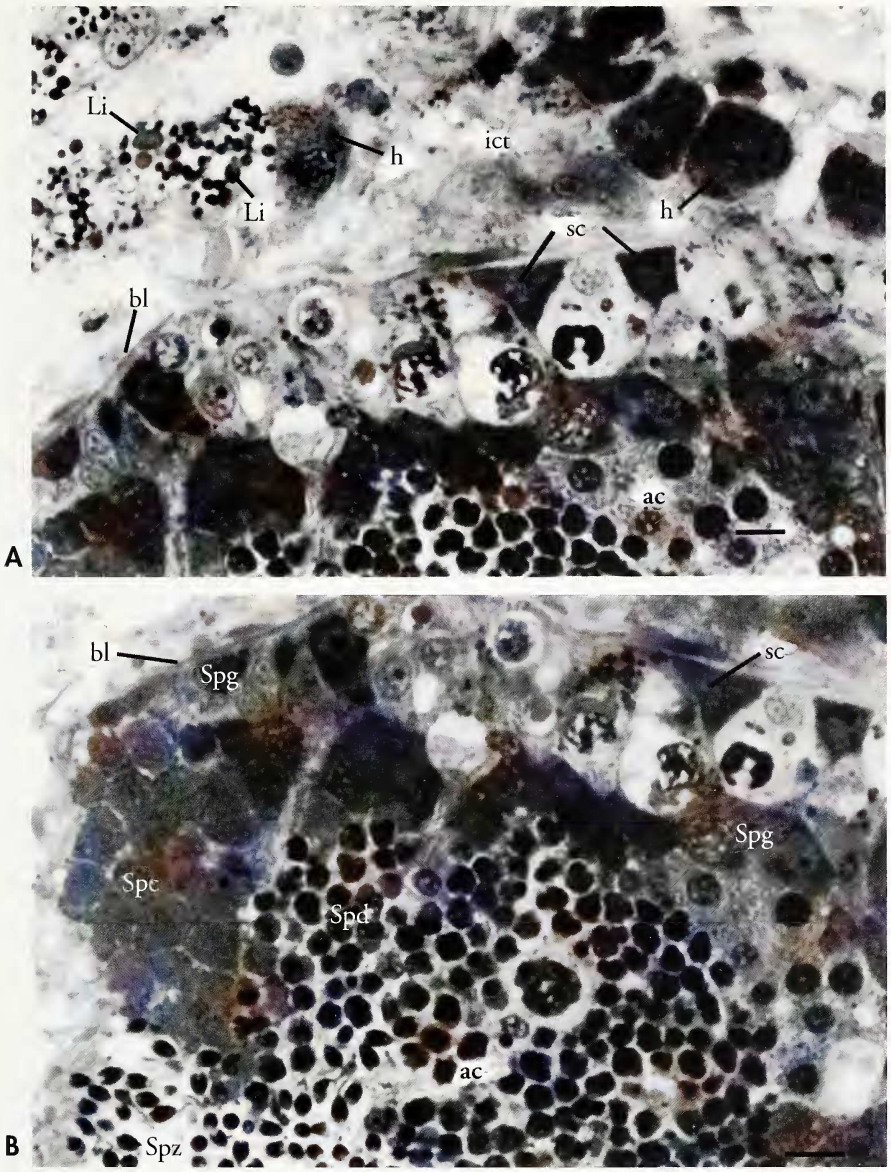


Figure 1. The male acini of *Perna perna*. A: semi-thin section showing vesicular cells in the interacinar connective tissue. These cells had two types of granules and lipid inclusions. B: semi-thin section of an acinus limited by a basal lamina. Sertoli-like cells and spermatogonia are located along its inner side. Abbreviations, ac: acinus; bl: basal lamina; h: hemocytes; ict: interacinar connective tissue; Li: lipid inclusions; sc: Sertoli-like cells; Spc: spermatocytes; Spd: spermatids; Spg: spermatogonia; Spz: spermatozoa; vc: vesicular cells. Scale bars, 10 µm.

Figura 1. Los ácinos macho de Perna perna. *A: sección semifina mostrando células vesiculares en el tejido conectivo entre ácinos. Estas células tenían dos tipos de gránulos y inclusiones de lípidos. B: sección semifina de un ácino limitado por una lámina basal. Células tipo Sertoli y espermatogonias están situados en su lado interno. Abreviaturas, ac: ácino; bl: lamina basal; h: hemocitos; ict: tejido conectivo interacinar; Li: inclusiones de lípidos; sc: células tipo Sertoli; Spc: espermatocitos; Spd: espermatidias; Spg: espermatogonia; Spz: espermatozoos; vc: células vesiculares. Escalas, 10 µm.*

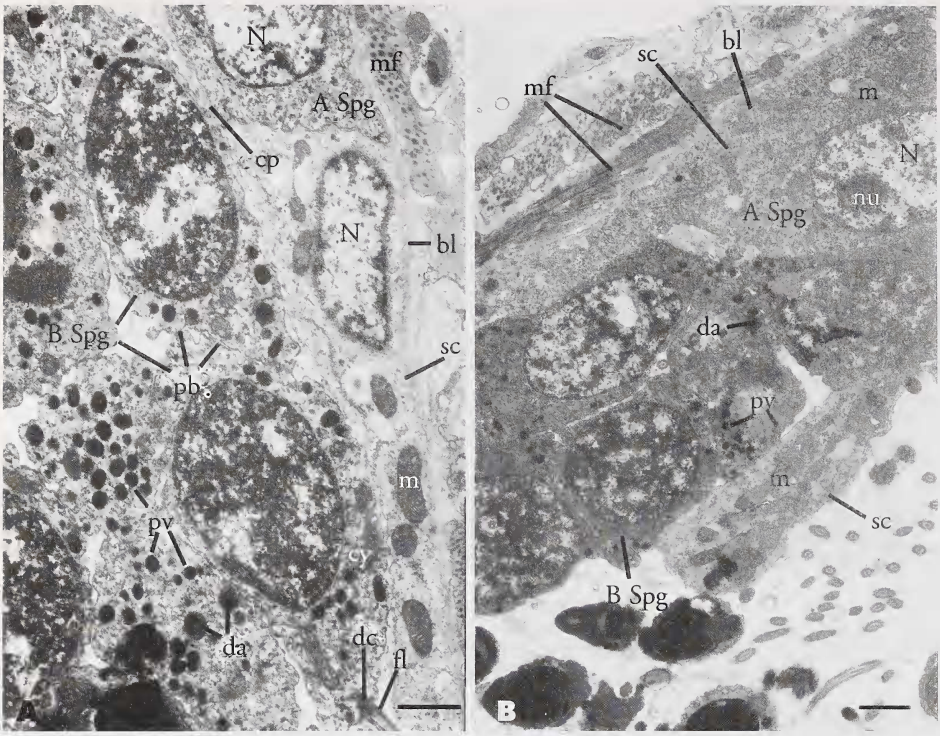
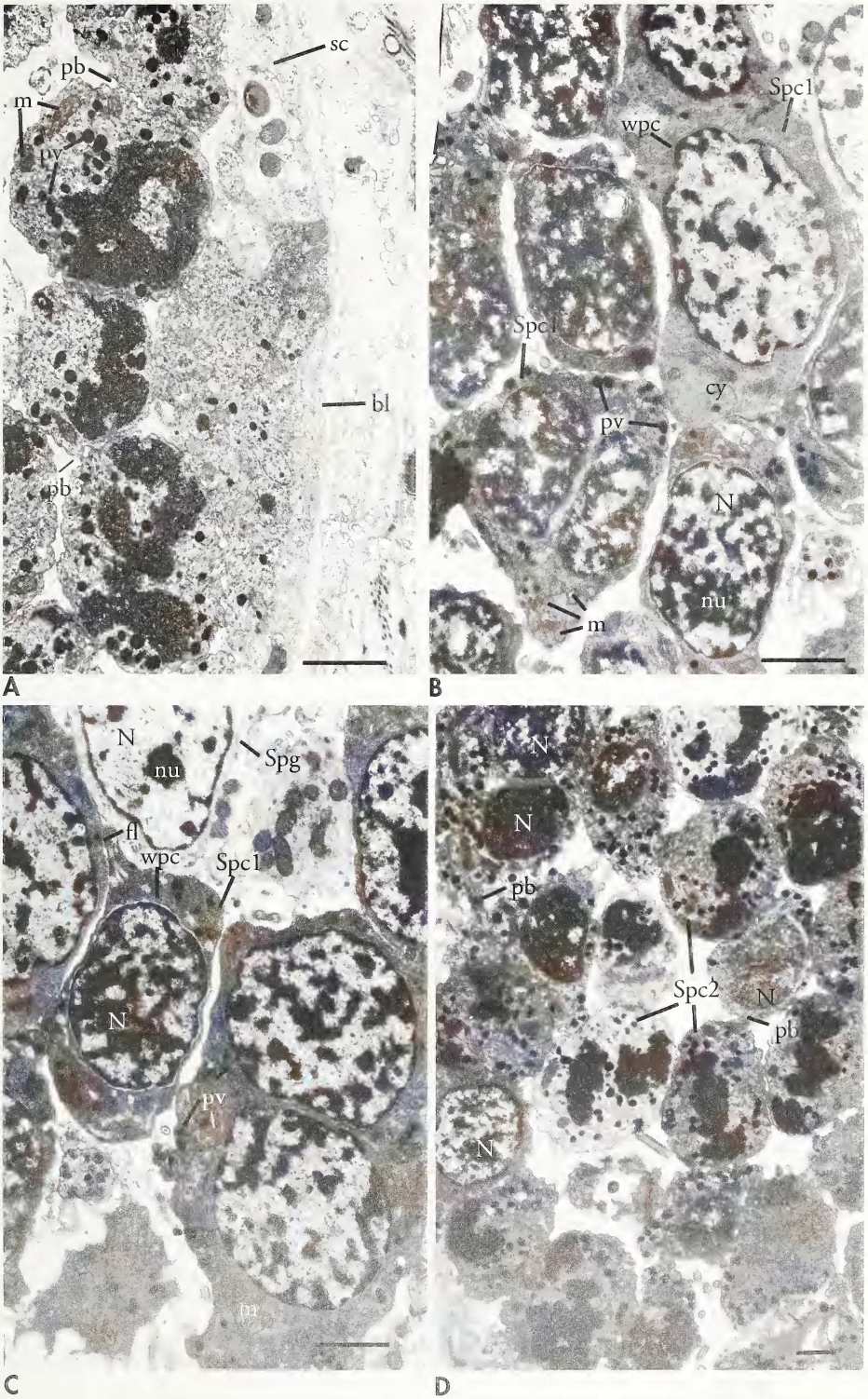


Figure 2. Ultrathin sections of early (A Spg) and late (B Spg) spermatogonia in *Perna perna*, separated from each other by cytoplasmic processes of Sertoli-like cells. Intercellular bridges between spermatogonia are frequent. Abbreviations: bl: basal lamina; cp: cytoplasmic processes of Sertoli-like cells; cy: cytoplasm; da: dense aggregates; dc: distal centrioles; fl: flagellum; m: mitochondria; mf: muscular fibers around the acinus; N: nucleus; nu: nucleolus; pb: intercellular bridges between spermatogonia; pv: proacrosomal vesicle; sc: Sertoli-like cells; Spg: spermatogonia. Scale bars, 5 µm.
Figura 2. Secciones ultrafinas de espermatogonias tempranas (A Spg) y tardías (B Spg) en Perna perna, *separadas unas de otras por extensiones citoplásmicas de células tipo Sertoli. Son frecuentes los puentes intercelulares entre espermatogonias. Abreviaturas: bl: lamina basal ; cp: extensiones citoplásmicas de células tipo Sertoli; cy: citoplasma; da: agregados densos; dc: centriolos distales; fl: flagelo; m: mitocondria; mf: fibras musculares rodeando el ácino; N: núcleo; nu: nucleolo; pb: puentes intercelulares entre espermatogonias; pv: vesícula proacrosomal; sc: células tipo Sertoli; Spg: espermatogonia. Escalas, 5 µm.*

(Right page) Figure 3. Ultrathin sections of spermatogonia and spermatocytes in *Perna perna*. A: B spermatogonia in metaphase. B, C: primary spermatocytes (Spc1) in prophase (pachytene stage), each showing a wide perinuclear cisterna. D: secondary spermatocytes (Spc2). Abbreviations, bl: basal lamina; cy: cytoplasm; fl: flagellum; m: mitochondria; N: nucleus; nu: nucleolus; pb: intercellular bridges between spermatogonia; pv: proacrosomal vesicle; sc: Sertoli-like cells; Spg: spermatogonia; wpc: wide perinuclear cisterna. Scale bar, 5 µm.
(Página derecha) Figura 3. Secciones ultrafinas de espermatogonias y espermatocitos de Perna perna. *A: espermatogonias B en metafase. B, C: espermatocitos primarios (Spc1) en profase (estadio paquiteno), cada uno mostrando una amplia cisterna perinuclear. D: espermatocitos secundarios (Spc2). Abreviaturas, bl: lámina basal ; cy: citoplasma; fl: flagelo; m: mitocondria; N: núcleo; nu: nucleolo; pb: puentes intercelulares entre espermatogonias; pv: vesícula proacrosomal; sc: células tipo Sertoli; Spg: espermatogonia; wpc: cisterna perinuclear ancha. Escalas, 5 µm.*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shaped, could reach up to 3.4 μm in length, and contained an axial rod from the apex to its basal part. Three types of material could be observed. An electron-lucent zone in the subacrosomal invagination and the center of the premembranoid sleeve could be observed, whereas two types of electron-dense materials were respectively present at the periphery of the apical part and in the base of the acrosome, the former material being denser than the latter. The axial rod passed outside the nucleus, from the apical to the posterior invaginations (Fig. 5A). At the base of the nucleus, five spherical mitochondria, of variable size (0.5-0.7 μm), surrounded the diplosome and constituted the middle piece (Fig. 5B). The two centrioles were situated in a perpendicular position and showed the classic nine triplets of microtubules (Fig. 5B). Some fibers, originating from the distal centriole and situated in a radiating position around it, formed a basal plate with the plasma membrane. Transversal sections of the axonemal complex of the flagellum (0.3 μm in diameter) showed the typical presence of 9 doublets along the periphery and a single central doublet. In this last zone, the cytoplasm was strongly reduced, thus increasing the nucleo-cytoplasmic ratio.

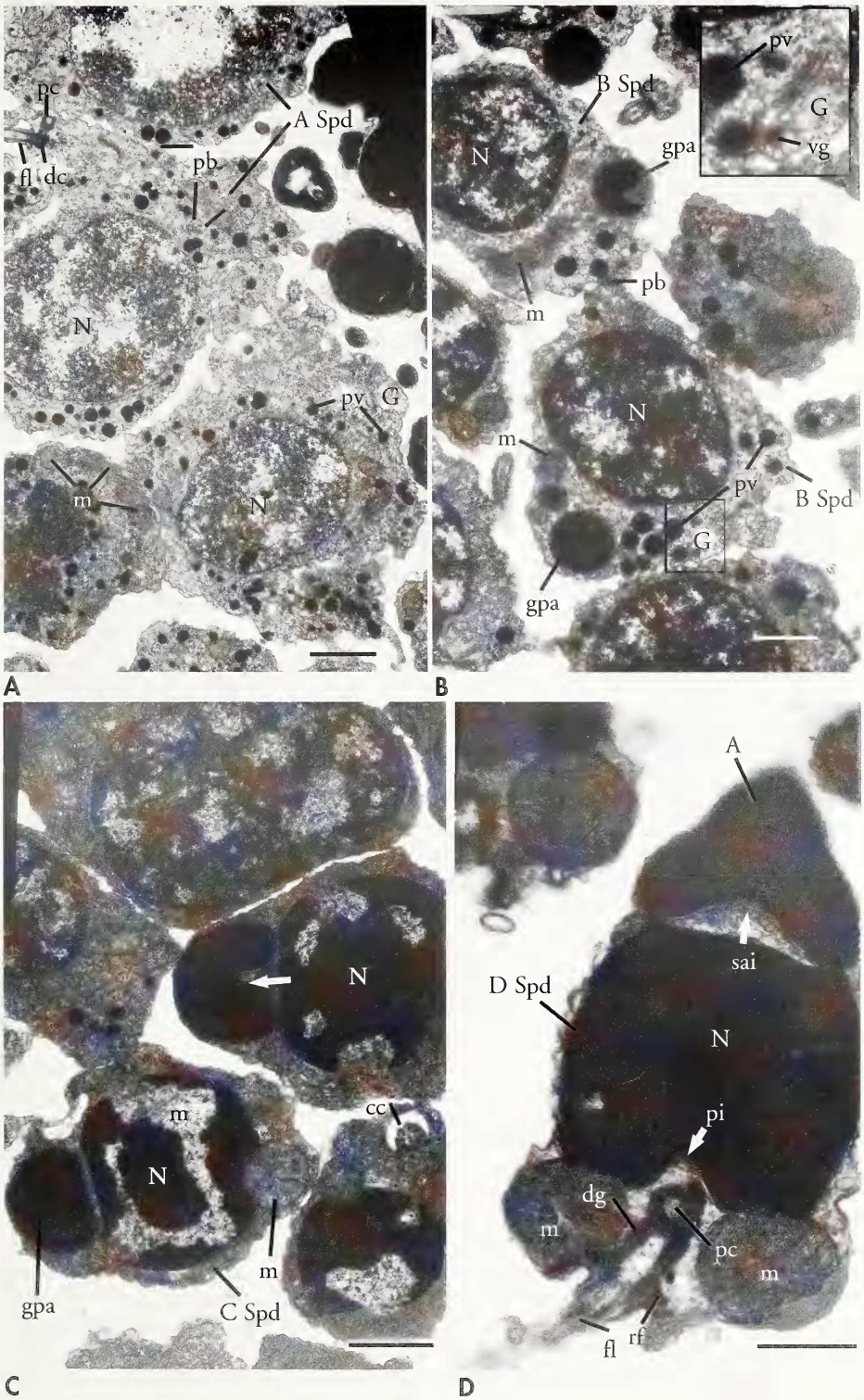
Abnormal spermatogenesis: Giant spermatogonia, each containing 2 to 5 nuclei (Fig. 6A), and binucleated spermatocytes were often found in December and March, respectively. In spermatids, lysis could affect the cytoplasm or the nucleus to finally yield to an extremely condensed nucleus or a thick nuclear envelope, respectively. At the end of spermatogenesis, the acrosome might lose its shape, curve, and even empty its material, thus giving an electron-lucent zone (Fig. 6B). Close to these abnormal cells, cytoplasmic inclusions with lipid inclusions and lysosomal membranes might be seen in the lumina of acini.

DISCUSSION

Most results on the spermatogenesis of *P. perna* are similar to those noted on the differentiation of male cells in other

(Right page) Figure 4. Ultrathin sections of spermiogenesis in *Perna perna*. A: a young spermatid (A Spd), with fusion of mitochondria and a Golgi complex showing synthetic activity. B: a proacrosomal granule and proacrosomal vesicles near the Golgi complex in an older spermatid (B Spd). C: a C spermatid (C Spd) with its nucleus showing a patchwork aspect. The proacrosomal granule is migrating to the anterior apex of the spermatid. D: an oldest spermatid (D Spd) showing the total condensation of the nucleus. The acrosome is at the cell apex. Abbreviations, A: acrosome; cc: centriolar complex; dc: distal centriole attached to the flagellum; fl: flagellum; G: Golgi complex; gpa: proacrosomal granule; ln: nuclear lacuna composed of heterochromatin; m: mitochondria; N: nucleus; pb: intercellular bridges; pc: proximal centriole; pi (white arrow): posterior invagination of the nucleus; pv: proacrosomal vesicles; rf: radiating fibers; sai (white arrow head): subacrosomal invagination of the nucleus; vg: Golgian vesicle. Scale bars, A-C: 2 μm; D: 1 μm.

(Página derecha) Figura 4. Secciones ultrafinas de espermiogénesis en Perna perna. *A: una espermatidia joven (A Spd), con fusión de mitocondrias y un complejo de Golgi mostrando actividad sintética. B: un gránulo proacrosomal y vesículas proacrosomales cerca del complejo de Golgi en una espermatidia más avanzada (B Spd). C: una espermatidia C (C Spd) cuyo núcleo muestra un aspecto en mosaico. El gránulo proacrosomal esta migrando hacia el ápice anterior de la espermatidia. D: una espermatidia de mayor edad (D Spd) mostrando la condensación total del núcleo. El acrosoma se sitúa en el ápice de la célula. Abreviaciones, A: acrosoma; cc: complejo del centriolo; dc: centriolo distal sujeto al flagelo; fl: flagelo; G: complejo de Golgi; gpa: gránulo proacrosomal ; ln: laguna nuclear compuesta por heterocromatina; m: mitocondria; N: núcleo; pb: puentes intercelulares; pc: centriolo proximal; pi (flecha blanca): invaginación posterior del núcleo; pv: vesículas proacrosomales; rf: fibras radiales; sai (cabeza de flecha blanca): invaginación subacrosomal del núcleo; vg: vesícula de Golgi. Escalas, A-C: 2 μm; D: 1 μm.*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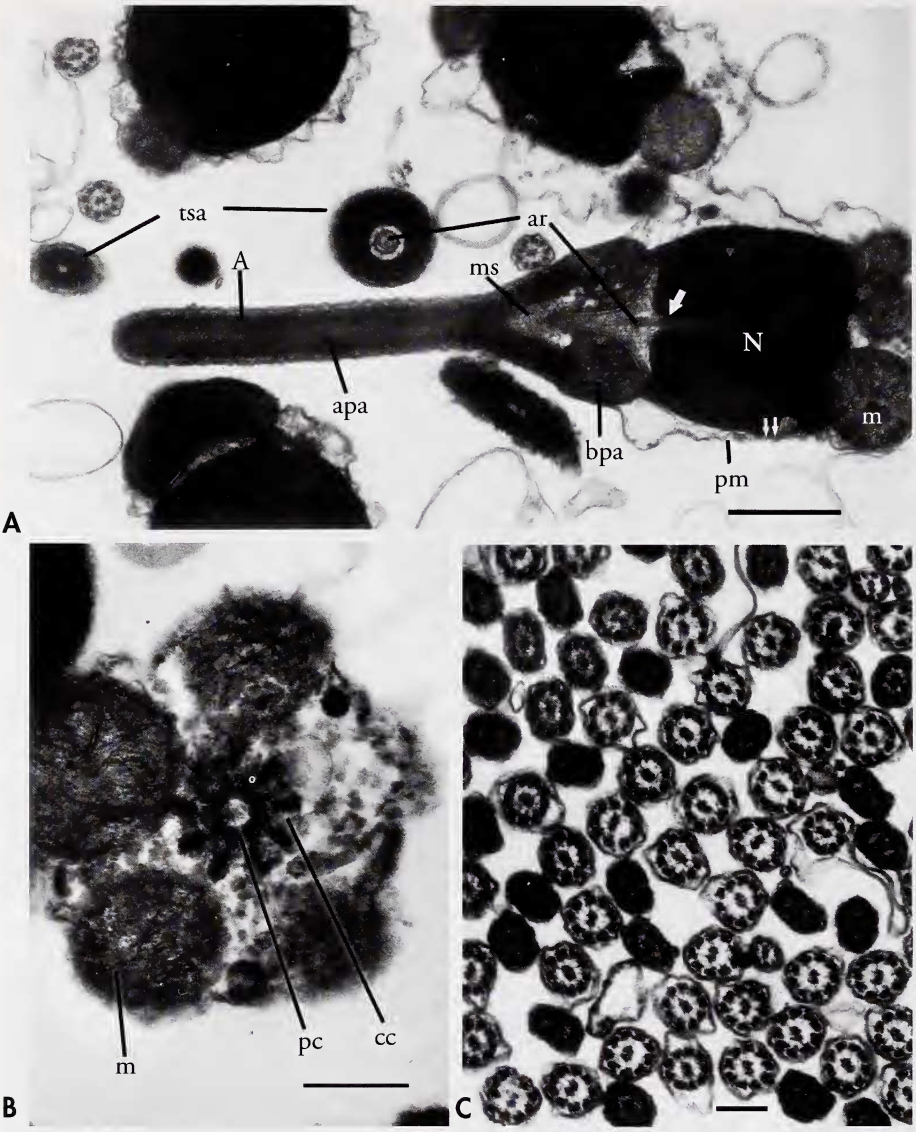

Figure 5. Mature spermatozoa (Spz) of *Perna perna*. A: longitudinal section, showing the barrel-like shaped nucleus and a greatly reduced cytoplasm (double white arrow). B: sagittal section of middle piece, showing five spherical mitochondria around the diplosome. C: transversal sections of flagella with nine peripheral doublets and a single central doublet of microtubules. Abbreviations, A: acrosome; apa: apical part of the acrosome; ar (white arrow): axial rod; bpa: basal part of the acrosome; cc: centriolar complex; m: mitochondria; ms: pre-membranoid sleeve; N: nucleus; pc: proximal centriole; pm: plasma membrane; tsa: transversal section of the acrosome. Scale bars, A, B: 2 µm; C: 0.5 µm.

Figura 5. Espermatozoos maduros (Spz) de Perna perna. *A: sección longitudinal, mostrando el núcleo en forma de barril y un citoplasma considerablemente reducido (doble flecha blanca). B: sección sagital de la parte mediana, mostrando cinco mitocondrias esféricas alrededor del diplosoma. C: secciones transversales de flagelos con nueve dobletes periféricos y un solo doblete cetral de microtúbulos. Abreviaturas, A: acrosoma; apa: parte apical del acrosoma; ar (flecha blanca): varilla axial; bpa: parte basal del acrosoma; cc: complejo del centriolo; m: mitocondria; ms: manga pre-membranóide; N: núcleo; pc: centriolo proximal; pm: membrana plasmática; tsa: sección transversal del acrosome. Escalas, A, B: 2 µm; C: 0,5 µm.*

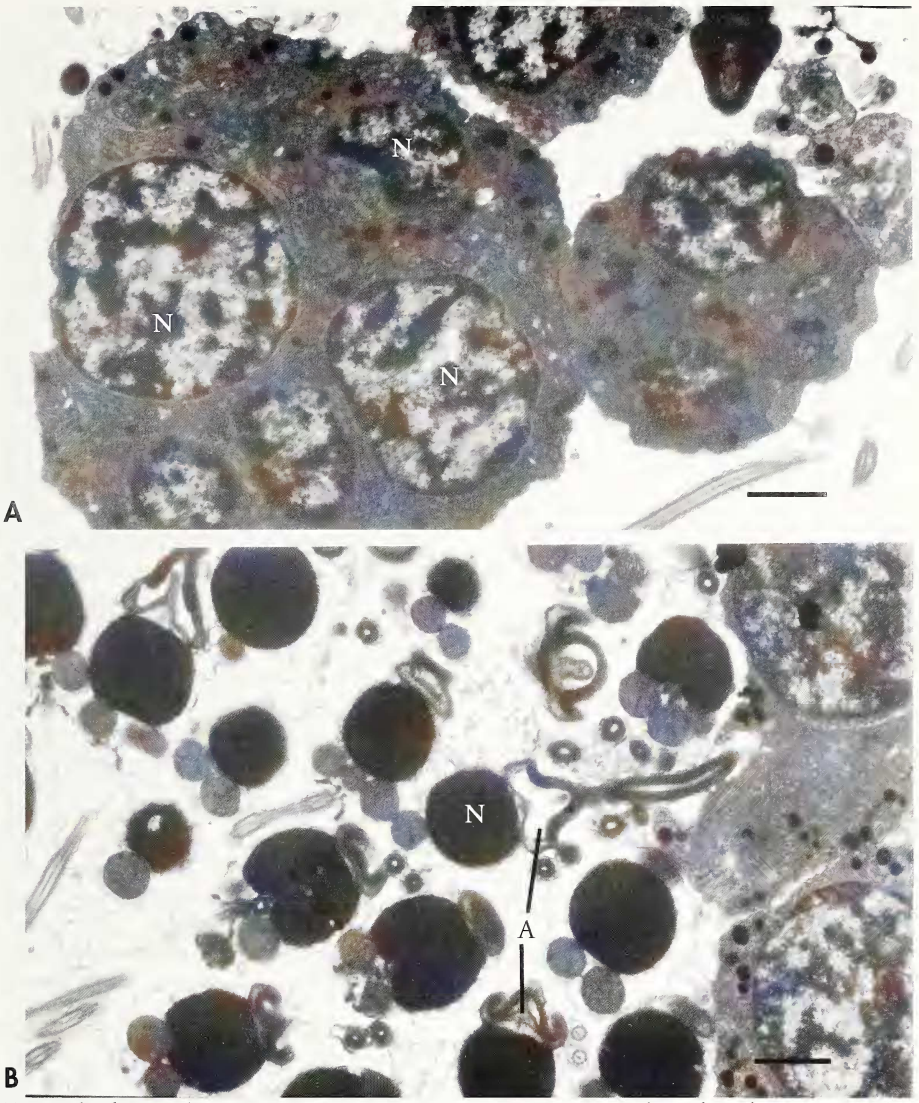

Figure 6. Abnormal spermatogenesis in *Perna perna*. A: a giant multinucleated spermatogonium. (B): acrosomal distortion, with emptying (black arrow) of the material (*). Abbreviations, A: acrosome; N: nucleus. Scale bars. 3 µm.

Figura 6. Espermatogenesis anómala en Perna perna. *A: una espermatogonia gigante multinucleada. B: distorsión acrosomal con escurrimiento (flecha negra) del material (*). Abreviaciones, A: acrosoma, N: núcleos. Escalas, 3 µm.*

bivalvia (LONGO AND DORNFELD, 1967; BERNARD AND HODGSON, 1985; HODGSON AND BERNARD, 1986A; REUNOV AND HODGSON, 1994; HEALY, 1996). All these reports demonstrated the existence of primitive spermatozoa (FRANZEN, 1955) in bivalvia. These primitive spermatozoa, also called aquasperm (ROUSE AND JAMIESON, 1987), are typical of invertebrate species which release their sperm in water. However, several points warrant special comments.

The barrel-like shape of the nucleus in the *P. perna* spermatozoon agreed with the report by BOUCART *ET AL.* (1965) on Brazilian *P. perna*, that of GARRIDO AND GALLARDO (1996) on *Perumytilus purpuratus*, or those of HODGSON AND BERNARD (1986a, b) and LONGO AND DORNFELD (1967) on *Mytilus edulis*. However, this shape was not the same for spermatozoa of other mytilid species (HODGSON AND BERNARD, 1986a, b; GARRIDO AND GALLARDO, 1996) and this discordance could be easily explained by the observations by POPHAM (1979). According to this author, in bivalvia, the shape of the sperm nuclei shows great variation in relation to species. The length of the nucleus (1.35 μm for the Moroccan *P. perna*) was close to the figures given by BOURCART *ET AL.* (1965) or by BERNARD AND HODGSON (1985) for two other populations of *P. perna* (1.40 and 1.50 μm, respectively). As the nuclei of spermatozoa in other species of Mytilidae was often longer (e.g., 1.76 μm for *M. galloprovincialis*: HODGSON AND BERNARD, 1986b), this parameter might be used to identify the spermatozoa of *P. perna* from those originating from other mytilid species.

The presence of an axial rod, in the nucleus of the spermatozoon, has already been reported for most Mytilidae (BOURCART *ET AL.*, 1965; LONGO AND DORNFELD, 1967; BERNARD AND HODGSON, 1985: HODGSON AND BERNARD 1986a, b; GARRIDO AND GALLARDO, 1996; LE PENNEC AND BENINGER, 1997; KAFANOV AND DROZDOV, 1998; REUNOV, AU AND WO, 1999). The axial rod is of great importance, as it takes part in the formation of the acrosomal filament (KAFANOV AND DROZDOV, 1998) and is projected ahead in the oocyte during the acrosomal reaction (REUNOV *ET AL.*, 1999). However, in our study, the rod did not have any transversal striation and longitudinal fibrils, as the cross-striation noted by BOURCART *ET AL.* (1965) in the spermatozoa of Brazilian *P. perna*, or that described by REUNOV *ET AL.* (1999) in *Perna viridis*, and this difference might be due to intraspecific variations between the different populations of this mussel.

Proacrosomal vesicles in early developmental stages of spermatogenesis were already described in all Mytilidae studied (THIELLEY, WEPPE AND HERBAUT, 1993; REUNOV AND HODGSON 1994; GAULEJAC, HENRY AND VICENTE, 1995) and this point cannot be used to discriminate the species of Mytilidae via the study of their spermatozoa. More interesting were the shape of the acrosome for *P. perna*, the existence of a premembranoid sleeve, and the presence of three types of materials therein. The funnel-like shape of this acrosome was greatly similar to that of *M. galloprovincialis* (BERNARD AND HODGSON, 1986b) and was considered by FRANZEN (1956) an adaptation allowing the penetration of the spermatozoon through the tertiary envelope surrounding the ovum. The presence of a premembranoid sleeve in the acrosome of *P. perna* has already been observed in the spermatozoon of *M. edulis* (LONGO AND DORNFELD, 1965). However, this sleeve was seen by BERNARD AND HODGSON (1985) only in spermatids of South African *P. perna*, whereas the corresponding spermatozoa did not have such structure. Another discordance can also be noted for the three types of material (2 electron-dense zones and a third more lucent) in the acrosome. If these three types were also observed in the acrosomes of *Pinctada margaritifera* (THIELLEY *ET AL.*, 1993) and of *Choromytilus chorus* (GARRIDO AND GALLARDO, 1996), two types of material (an electron-dense zone and a more lucent) were only described in the acrosomes of South African *P. perna* (BERNARD AND HODGSON, 1985) as well as in those of other mytilids (POPHAM, 1979; HODGSON AND BERNARD, 1986a, b; GAULEJAC *ET AL.*, 1995). Moreover, a reticular structure in the dense zone of the acrosome apex was described in the Brazilian *P. perna*, whereas it was not found in the Moroccan mussels. To explain these three differences, the more likely hypothesis is to consider them intraspecific variations, probably linked to the presence of local subspecies of *P. perna* in Brazil, Morocco, and South Africa.

The structure of the *P. perna* flagellum, with 9 peripheral doublets and a single central doublet, was similar to that noted for the tails of spermatozoa which fertilized ova in sea water. The presence of short flagella in some B spermatogonia and primary spermatocytes was already reported by REUNOV AND HODGSON (1994) in other mytilids. The radiating fibers around the distal centriole were also described in the spermatozoa of several Mytilidae (REUNOV AND HODGSON, 1994) and of *Haliotis tuberculata* (BELHSEN, 2000). According to GRACIA BOZZO, RIBES, SAGRISTA, POQUET AND DURFORT (1993), such radiating fibers would allow control of the motility, direction, and speed of gametes.

Abnormal cells noted in the spermatogenesis of *P. perna* were also observed in other bivalvia by DORANGE AND LE PENNEC (1989), GAULEJAC *ET AL.* (1995), or THIELLEY *ET AL.* (1993). Indeed, the formation of giant cells, a nuclear or cytoplasmic lysis, and changes in the shape of the acrosome might be the result of accidental deviations during spermatogenesis. However, as many abnormal cells were seen in the lumina of acini, it can be assumed that these cells would be rapidly eliminated via their being carried to the male genital orifice.

In conclusion, the spermatogenesis of *P. perna* was similar to differentiations of male cells described in other bivalvia. However, several differences could be noted. The length of the nucleus, the premembranoid sleeve, and the presence of three types of material in the acrosome may be, in our opinion, efficient criteria i) to separate the Moroccan populations of *P. perna* from similar populations living in another countries, and ii) to identify the communities of this species from other Mytilidae and, in particular, from those of *M. galloprovincialis* which also lives along Moroccan coasts.

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